Effect of Heat Treating Alfalfa Hay on Chemical Composition and Ruminal In Vitro Protein Degradation[1]

J. H. YANG,[2] G. A. BRODERICK,[3] and R. G. KOEGEL
Agricultural Research Service, USDA
US Dairy Forage Research Center
1925 Linden Drive West
Madison, WI 53706

ABSTRACT

Conventional (unshredded) and shredded alfalfa hays were heated in either a forced-air oven or a steam pressure cooker at different times and temperatures to determine the effect of heat treatment on chemical composition and ruminal protein degradability. Rates of protein degradation and extents of protein escape were estimated using a ruminal inhibitor in vitro system. Both rates and extents were corrected for the proportion of total N in ADIN. Estimated net protein escape (total escape minus ADIN-bound CP) of unshredded and shredded hays was increased by oven or steam heating. Optimal oven treatments, as indicated by the greatest increase in net protein escape, were 120 min at 150°C and 60 min at 160°C. Net protein escapes of shredded hay were greater than unshredded hay when neither was heated and when hays were heated to the same extent. Equivalent protein protection was obtained by oven heating for 120 min at 140°C, 60 min at 150°C, and 30 min at 160°C, which gave net protein escapes of 55, 54, and 54% for shredded hay and 44, 45, and 43% for unshredded hay, respectively. Similar protein protection was obtained at lower temperatures and shorter times with steam heating. Nearly optimal net protein escapes were obtained with steam treatment for 30 to 120 min at 100°C or for 15 min at 110 or 120°C. Oven heating and, especially, steam heating of hays for the longer times increased ADIN to very high percentages. These data indicated that moderate heat treatment of alfalfa hay reduces ruminal protein degradation and improves protein utilization.

(**Key words**: alfalfa hay, protein utilization, heat treatment)

Abbreviation key: **TN** = total N; **UIP** = undegraded intake protein.

INTRODUCTION

Alfalfa hay is a major source of dietary protein for lactating dairy cows in some regions; however, extensive degradation in the rumen may reduce protein utilization (2). Heat treatment of cottonseed meal (21), soybean meal (5, 23), and whole soybeans (8, 25) has enhanced protein resistance to ruminal degradation and improved animal performance. Heating alfalfa also may improve protein utilization. Replacing soybean meal with protein from heat-processed alfalfa appeared to reduce ruminal degradation and increase intestinal AA absorption (17); partial replacement of dietary alfalfa silage with dehydrated alfalfa increased milk yield (19). Lambs fed alfalfa hay dehydrated at 120 or 145°C had improved digestibility and growth compared with those of lambs fed barn-dried alfalfa hay (11). However, overheating of alfalfa reduced apparent digestibility of DM, N, N-free extract, and ADF (11, 13, 28). Shredding of alfalfa enhanced drying rates (14) and improved in vivo fiber digestibility (15); field shredding of al-

Received May 18, 1992.
Accepted September 17, 1992.

[1]Mention of a trademark or proprietary product in this paper does not constitute a guarantee or warranty of the product by the USDA or the Agricultural Research Service and does not imply its approval to the exclusion of other products that also may be suitable.

[2]Present address: Guangxi Animal Husbandry Research Institute, Nanning, Guangxi, People's Republic of China.

[3]Corresponding author.

falfa may become a practical harvesting method.

The objectives of this study were 1) to determine the effects on chemical composition of heating of both conventional (unshredded) and shredded alfalfa hay; and 2) to identify the optimal heating conditions, using both dry, forced-air heating and steam heating to maximize ruminal escape of alfalfa protein with minimal loss from overheating. An inhibitor in vitro system (3) was used to estimate rates and extents of ruminal protein degradation; the proportion of total N (TN) in ADIN (26, 27) was used to assess overheating. The most appropriate heating method was to be used to reduce ruminal degradation of protein in alfalfa hay fed to lactating cows (6).

MATERIALS AND METHODS

All of the first-cutting alfalfa hay used in this study was from the same field and was cut with a sickle bar mower without crimping as long forage at early bloom stage on June 15, 1988. A portion (unshredded control) was allowed to sun cure on black plastic to about 15% DM. The remaining forage was taken immediately after cutting to the laboratory, where it was shredded and formed into mats using the stationary macerator described by Koegel et al. (15). The mats also were allowed to sun cure on black plastic to about 15% DM at the same time in the same location as the unshredded control. After drying, both hays were chopped to a nominal length of 7.5 cm and stored at room temperature (22°C) in plastic bags.

Alfalfa hay was oven heated using a forced-air oven by placing about 120 g of each chopped alfalfa hay into a tray (70 × 25 × 2.5 cm) with a wooden frame and wire screen at the top and bottom. One layer of cheesecloth was spread over the bottom screen to keep the small hay particles from falling through the screen during heating. Hay samples were heated in the oven at 130, 140, 150, and 160°C for 15, 30, 60, 90, and 120 min. Each batch of hay was heated separately; hay was placed into the preheated oven and timed from the point at which oven temperature had returned to the original setting (about 2 to 3 min). Hay was allowed to cool to room temperature (22°C) immediately after completing heat treatment.

Alfalfa hay was steam heated by wrapping 120-g samples of each chopped hay in one layer of cheesecloth and suspending them in a rack within a steam pressure cooker. About 2 cm of water had been added to the cooker prior to the forage; care was taken so that hay did not come into direct contact with the water during the heating process. The pressure cooker was heated on a gas stove at 100, 110, 120, and 130°C for 15, 30, 60, and 120 min. Temperatures within the cooker were maintained by four separate pressure relief valves set at 0, 42.1, 97.2, and 168.9 kPa. Each batch of hay was heated separately; timing was from the point at which the cooker reached the desired temperature (about 3 to 5 min). Hay was allowed to cool to room temperature (22°C) immediately after completing heat treatment.

Following heating, chopped hays were ground with a Wiley mill (Arthur H. Thomas, Philadelphia, PA) through a 1-mm screen and analyzed in triplicate for TN by Kjeldahl, using a copper digestion catalyst (Kjeltabs®; Tecator Inc., Herndon, VA) for DM at 105°C (1), for NDF and ADF (18), and for the proportion of TN present as ADIN (12). Duplicate samples (.250 g) were extracted with 25 ml of distilled water with swirling for 60 min at room temperature (22°C); extracts were filtered through Whatman number 1 filter paper (Whatman, Clifton, NJ), and filtrates were analyzed for total reducing sugars using glucose as standard (24). Each hay also was assayed for fractions degraded at 0 h (fraction A) and potentially degradable (fraction B) and for rate of ruminal protein degradation using the inhibitor in vitro system described by Broderick (3), except that incubations were conducted for 0 to 2 h in 50-ml centrifuge tubes and were stopped by addition of only TCA. Hay samples were incubated as two separate sets, one each for oven-heated and steam-heated hays; each set of incubations was replicated three times. Casein was included as a standard protein in each incubation. Degradation rates were corrected for unavailable N (fraction C), assuming that ADIN was equivalent to this fraction (3). Estimated net extent of ruminal protein escape (i.e., corrected for unavailable N) was computed using the equation:

$$\text{net ruminal protein escape (\%)} = B \times [k_p/(k_d + k_p)]$$

where $B = [1 - (A + C)] \times 100$; k_d is the rate of ruminal protein degradation; and k_p, the

TABLE 1. Effect of shredding alfalfa hay on mean composition and ruminal in vitro protein degradability.[1]

Item	Unshredded	Shredded	SE	$P > F$
ADF, % of DM	34.9	32.6	.6	.010
NDF, % of DM	44.4	42.5	.7	.144
Reducing sugars, mmol/100 g of DM	22.8	20.5	.4	.010
TN,[2] % of DM	2.86	2.99	.04	.088
ADIN (C), % of TN	5.9	5.3	.1	.003
0-h Degraded protein (A), % of TN	11.6	6.0	.3	<.001
Degradable protein (B), % of TN	82.6	88.7	.2	<.001
Degradation rate (k_d),[3] /h	.184	.153	.007	.033
Net ruminal escape,[4] % of TN	21.0	25.4	.8	.008

[1]Degradable protein (B), percentage of TN = [1 − (A + C)] × 100, where A = fraction degraded protein at 0 h (i.e., N as NH_3 and total AA), and C = ADIN (fraction undegradable N).

[2]TN = Total N.

[3]Fractional ruminal degradation rates were determined in the inhibitor in vitro system (3) assuming that the ADIN fraction was undegradable.

[4]Percentage of net ruminal protein escape = B × [k_p/(k_p + k_d)], where k_p was assumed to be = .06/h (3).

ruminal passage rate, was assumed to be equal to .06/h.

The general linear models of SAS (20) were used for ANOVA and regression analyses of contents of NDF, ADF, reducing sugars, and TN; proportions of TN in ADIN and in fractions A and B; protein degradation rates; and net protein escapes. Separate analyses were conducted on the two types of heat treatment (oven or steam); models included treatment (shredded or unshredded), temperature, time, and replicate plus the interactions of time by temperature and time by treatment. When significant effects ($P < .01$) that were due to temperature were detected, separation of means was by least significant difference at $P = .05$ (22). Additional ANOVA were conducted to assess the significance of alfalfa treatment (shredded or unshredded), or conducted among hays treated at specific times and temperatures by oven or steam heating, on NDF, reducing sugars, ADIN, degradation rate, and net protein escape. Also, regressions on reducing sugar concentration of ADIN, degradation rate, and net protein escape were conducted.

RESULTS AND DISCUSSION

The inhibitor in vitro system (3) used in these studies was developed to estimate rates and extents of ruminal protein degradation. This method has been used to determine optimal heating conditions for whole soybeans (9, 10); a similar procedure was used earlier to determine the optimal autoclaving time for cold-extracted cottonseed meal (4). Overheating of alfalfa was quantified from proportion of TN in ADIN (26, 27). The inhibitor in vitro system gave mean ruminal protein escapes of 28.8% (total escape) and 23.2% (net escape; total escape minus ADIN CP) for unshredded control and shredded hays (Table 1). These values may be considered to be estimates of undegraded intake protein (**UIP**). The NRC (16) reported UIP for alfalfa hay of 28%. Compared with unshredded hay, shredded hay had lower ADF, reducing sugars, ADIN, degraded protein at 0 h (fraction A), and degradation rate and higher degradable protein (fraction B) (Table 1). Increased net ruminal protein escape was the result of larger fraction B and lower degradation rate. Both hays were dried under the same conditions. However, greater solar heat absorption, in shredded hay, because of its darker color (15) may have increased drying temperatures, increased reaction of reducing sugars with protein (26, 27), and reduced protein degradability. More rapid drying of shredded hay (15) may account for lower fraction A because DM concentrations at which autolysis of forage protein ceases were reached more quickly. The trend ($P = .088$) for higher TN content in shredded hay may have been due to reduced leaf loss. Overall mean degradation rate was lower, and net ruminal

TABLE 2. Mean composition and ruminal in vitro protein degradability of unshredded (control) and shredded alfalfa hays that were either oven or steam heated.

Heat treatment	Forage treatment	NDF	Reducing sugars	ADIN	Degradation rate	Net escape
		(% DM)	(mmol/100 g of DM)	(% of TN[1])	(/h)	(% of TN)
Oven	Unshredded	50.7	8.7	10.6	.072	38.7
	Shredded	50.1	8.4	11.0	.051	47.6
	$P > F$	.003	.012	<.001	<.001	<.001
Steam	Unshredded	54.4	8.2	13.7	.063	38.0
	Shredded	55.2	8.3	19.9	.051	41.0
	$P > F$	.029	.261	<.001	<.001	.001

[1]TN = Total N.

protein escape also was greater, in shredded than in unshredded hay when both were heated under the same conditions (Table 2). Although differences in net protein escape tended to narrow with steam treatment because of excessive ADIN formation, the greater estimated UIP in heated shredded hay probably reflects differences already present before heating.

Mean composition of unshredded and of shredded hay over all oven heating times are in Table 3. Concentrations of ADF, NDF, and ADIN were elevated significantly with in-

TABLE 3. Effect of heat treating alfalfa hay in a forced-air oven at 130 to 160°C on mean composition and ruminal in vitro protein degradability.[1]

Item	Unheated	Temperature 130°C	140°C	150°C	160°C	SE	Regression[2]
ADF, % of DM	34.2	34.3^{c}	34.3^{c}	36.0^{b}	38.1^{a}	.1	L*** Q***
NDF, % of DM	42.9	47.0^{d}	48.4^{c}	51.5^{b}	54.5^{a}	.2	L*** Q***
Reducing sugars, mmol/100 g of DM	20.6	13.3^{a}	9.0^{b}	7.0^{c}	5.1^{d}	.1	L*** Q***
TN,[3] % of DM	2.91	3.00^{b}	3.05^{a}	3.02^{ab}	2.94^{c}	.02	L**
ADIN (C), % of TN	5.5	6.4^{d}	8.2^{c}	11.1^{b}	17.4^{a}	.2	L*** Q*** C*
0-h Degraded protein (A), % of TN	9.2	6.6^{a}	6.0^{ab}	5.8^{b}	5.2^{c}	.2	L***
Degradable protein (B), % of TN	85.3	87.0^{a}	85.8^{b}	83.1^{c}	77.5^{d}	.2	L*** Q***
Degradation rate (k_d),[4] /h	.193	.091^{a}	.067^{b}	.052^{c}	.036^{d}	.002	L*** Q***
Net ruminal escape,[5] of TN	20.6	35.8^{d}	41.9^{c}	45.8^{b}	49.3^{a}	.6	L*** Q**

^{a,b,c,d}Temperature means with differing superscripts differ ($P < .05$). Probability of significant temperature effect was $P < .001$ for all traits.

[1]Degradable protein (B), percentage of TN = $[1 - (A + C)] \times 100$, where A = fraction degraded protein at 0 h (i.e., N as NH_3 and total AA), and C = ADIN (fraction undegradable N).

[2]L, Q, and C = Significant linear, quadratic, or cubic regressions on time, respectively.

[3]TN = Total N.

[4]Fractional ruminal degradation rates were determined in the inhibitor in vitro system (3) assuming that ADIN fraction was undegradable.

[5]Percentage of net ruminal protein escape = $B \times [k_p/(k_p + k_d)]$, where k_p was assumed to be = .06/h (3).

*$P < .05$.

**$P < .01$.

***$P < .001$.

creased temperatures. These changes were accompanied by decreased reducing sugars, fraction B (because of elevated ADIN), and degradation rate and increased net ruminal protein escape (Table 3). For example, mean degradation rate for both unshredded and shredded hays for all times at 160°C was .037 versus .193/h for unheated hays, and mean net ruminal protein escape was 49 versus 21% for unheated hays (Table 3). Optimal treatments for roasted soybeans gave degradation rates and protein escapes, estimated using the same ruminal in vitro system, of about .030/h and 60% (9). Linear and quadratic regressions on time of heating were significant for all variables except TN and fraction A, for which only linear time effects were significant; ADIN also had a significant cubic regression on time (Table 3). Graphical presentation of the time-temperature relationships with oven heating for all variables except ADF and TN are in Figure 1.

Mean composition changes averaged for unshredded and shredded hay over all heating times with steam treatment are in Table 4. Generally, effects of steam heating were similar to those of oven heating: significant elevation of ADF, NDF, and ADIN; decreased reducing sugars, fractions A and B (because of elevated ADIN), and degradation rates; and increased net ruminal protein escapes (Table 4). Significant linear regressions on time of heating were significant for all variables except degradation rate, which did not alter with time or temperature. Steam heating reduced protein degradation rate from .145/h to an overall average of .057/h. Significant quadratic (reducing sugar, ADIN, and fraction B) and cubic (reducing sugars) regressions on time also were observed (Table 4). Graphical presentation of the time-temperature relationships with steam heating for all variables except ADF and TN is in Figure 2.

The magnitude of increase in fiber was greater with steam heating, despite lower temperatures, than with oven heating. For example, mean NDF at 100°C with steam treatment (Table 4) was about equal to mean NDF at 160°C of oven heating (Table 3). The in-

TABLE 4. Effect of heat treating alfalfa hay with steam at 100 to 130°C on mean composition and ruminal in vitro protein degradability.[1]

Item	Unheated	Temperature 100°C	110°C	120°C	130°C	SE	Regression[2]
ADF, % of DM	33.2	35.6^{c}	37.2^{b}	38.9^{a}	39.7^{a}	.3	L***
NDF, % of DM	44.0	53.5^{b}	55.4^{a}	55.1^{a}	55.3^{a}	.3	L***
Reducing sugars, mmol/100 g of DM	22.7	9.6^{a}	8.6^{b}	7.5^{c}	7.4^{c}	.1	L*** Q* C**
TN,[3] % of DM	2.93	2.90^{b}	2.93^{b}	3.01^{a}	3.01^{a}	.03	L*
ADIN (C), % of TN	5.7	7.1^{d}	10.8^{c}	20.0^{b}	29.5^{a}	.4	L*** Q***
0-h Degraded protein (A), % of TN	8.4	8.1^{a}	7.3^{ab}	6.6^{bc}	6.2^{c}	.3	L**
Degradable protein (B), % of TN	86.0	84.9^{a}	81.9^{b}	73.4^{c}	64.3^{d}	.4	L*** Q***
Degradation rate (k_d),[4] /h	.145	.056	.060	.055	.058	.003	
Net ruminal escape,[5] % of TN	25.8	44.3^{a}	41.7^{b}	38.8^{c}	33.1^{d}	.9	L***

^{a,b,c,d}Temperature means with differing superscripts differ ($P < .05$). Probability of significant temperature effect was $P < .001$ for all traits except TN ($P = .005$) and degradation rate ($P = .765$).

[1]Degradable protein (B), percentage of TN = [1 − (A + C)] × 100, where A = fraction degraded protein at 0 h (i.e., N as NH_3 and total AA), and C = ADIN (fraction undegradable N).

[2]L, Q, and C = Significant linear, quadratic, or cubic regressions on time, respectively.

[3]TN = Total N.

[4]Fractional ruminal degradation rates were determined in the inhibitor in vitro system (3) assuming that the ADIN fraction was undegradable.

[5]Percentage of net ruminal protein escape = B × [$k_p/(k_p + k_d)$], where k_p was assumed to be = .06/h (3).

*$P < .05$.

**$P < .01$.

***$P < .001$.

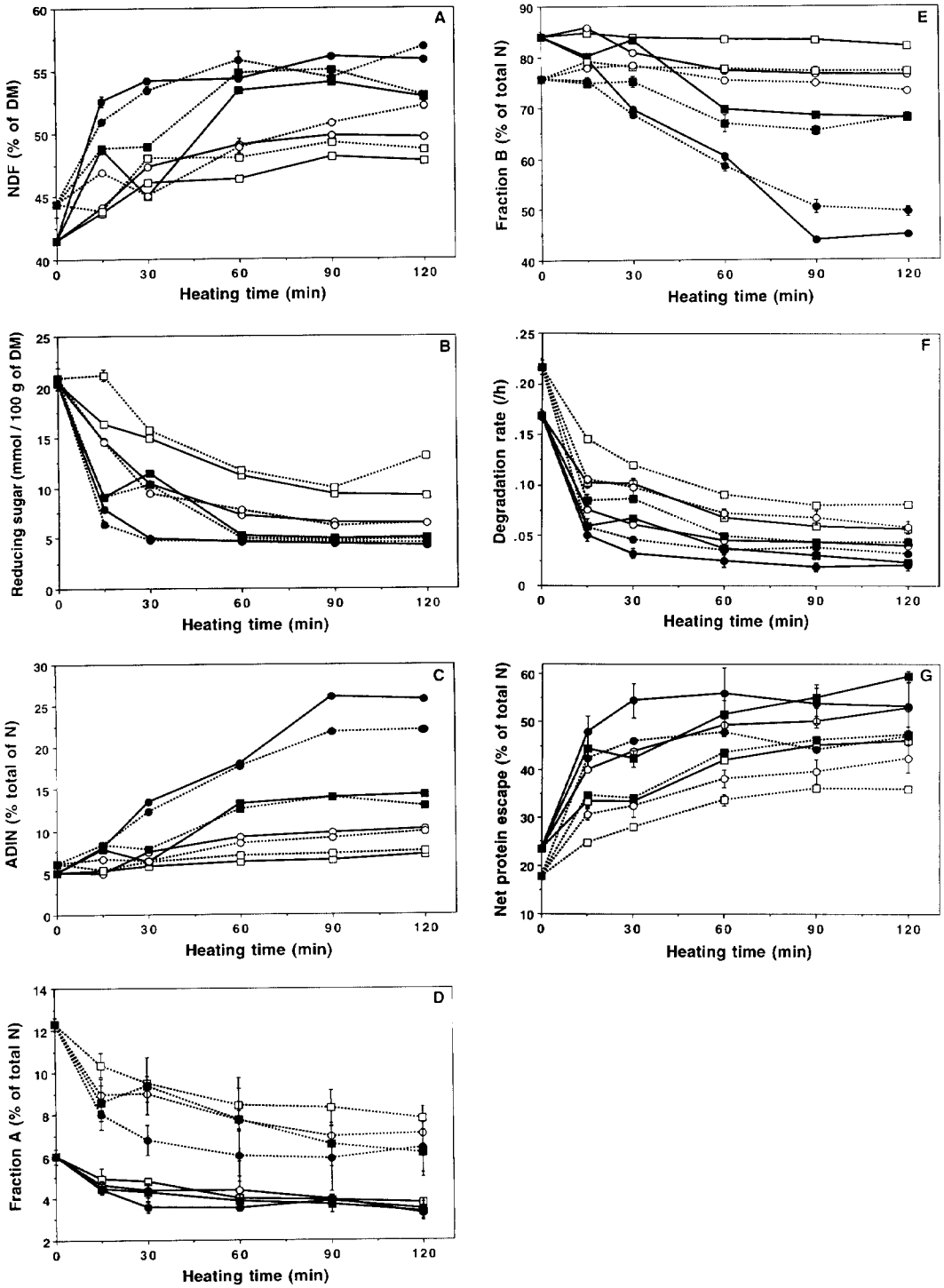

Figure 1. Effect of oven heating of unshredded (control; - - - -) and shredded (—) alfalfa hay on A) the concentrations of NDF and B) concentrations of reducing sugars, C) proportions of total N in ADIN, D) fraction A (0-h degraded protein) and E) fraction B (degradable protein), F) ruminal in vitro protein degradation rate, and G) net protein escape (total escape minus ADIN CP). Temperatures were 130 (□), 140 (○), 150 (■), and 160°C (●). Vertical lines represent ± 1 SE.

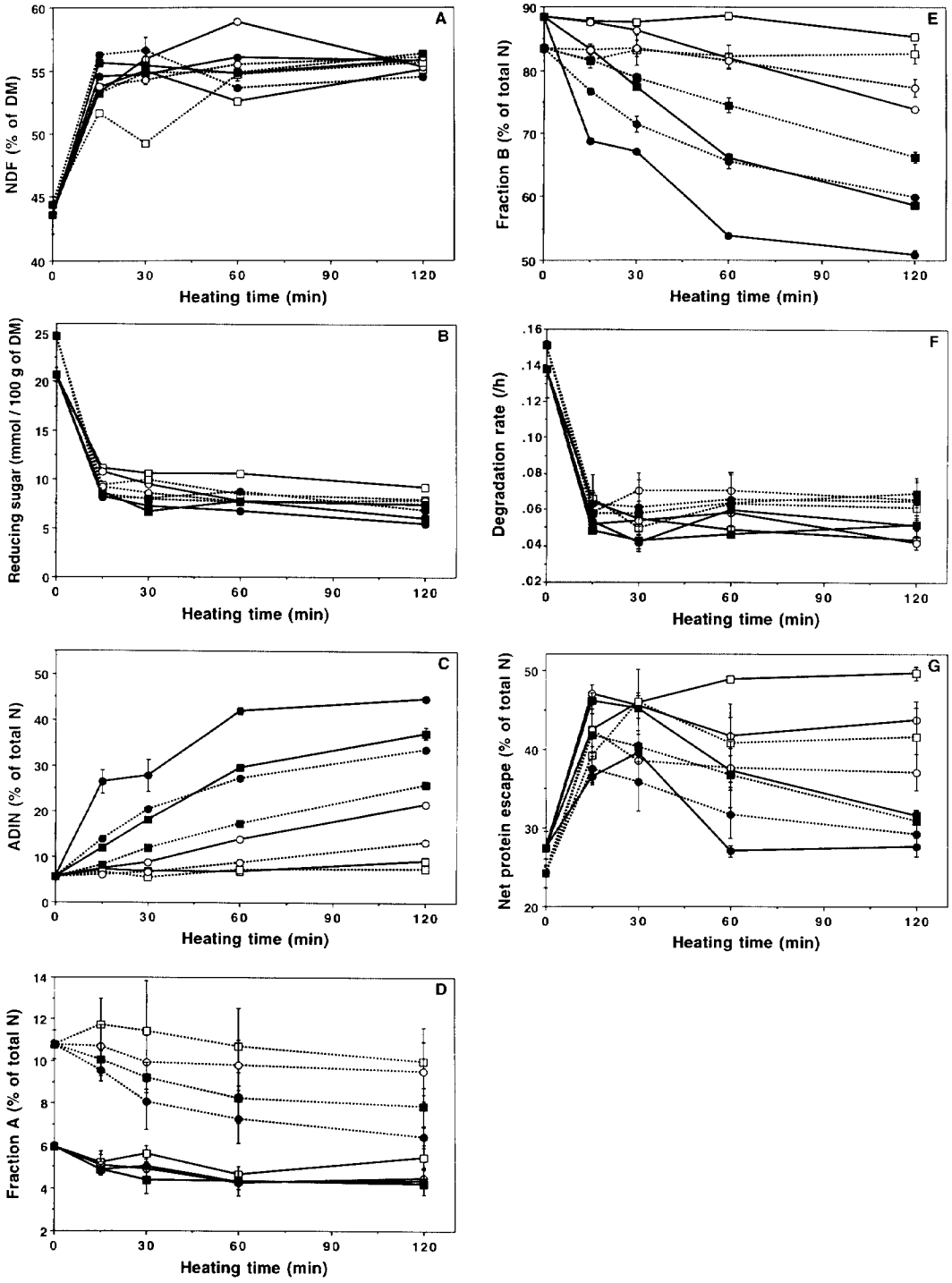

Figure 2. Effect of steam heating of unshredded (control; ----) and shredded (—) alfalfa hay on A) the concentrations of NDF and B) concentrations of reducing sugars, C) proportions of total N in ADIN, D) fraction A (0-h degraded protein) and E) fraction B (degradable protein), F) ruminal in vitro protein degradation rate, and G) net protein escape (total escape minus ADIN CP). Temperatures were 100 (□), 110 (○), 120 (■), and 130°C (●). Vertical lines represent ± 1 SE.

creases, over unheated samples, in mean ADIN at 160°C (oven) and 130°C (steam) were 12 and 24 percentage units (Tables 3 and 4, respectively). With oven heating, ADIN concentration increased slowly at 130 and 140°C, did not exceed 14% of TN at 150°C, and only rose above 20% of TN after 90 and 120 min at 160°C (Figure 1C). Although ADIN remained less than 10% of TN at 100°C, steam heating increased ADIN to excessively high percentages at temperatures greater than 110°C (Figure 2C). Slower reaction rates with hot air rather than with steam pressure also were illustrated by the more gradual decline in degradation rates with oven heating (Figure 1F) than with steam heating (Figure 2F). The range in degradation rates after 15 min of oven heating was .05 to .15/h but only .05 to .07/h with steam treatment. Heat transfer from water vapor with heating under steam pressure appears to be more effective than with hot air heating (21). Although mean net protein escape increased with temperature when hay was oven heated (Table 3), mean net protein escape declined at steam temperatures greater than 100°C because of substantial elevation in ADIN (Table 4).

Protection of alfalfa hay protein from ruminal degradation with heat treatment may be mediated partly by the Maillard reaction between reducing sugars and protein (26, 27). As with protein degradation rate, reducing sugar concentrations generally declined with increased temperatures (Tables 3 and 4) and heating times (Figures 1B and 2B). Although overall regressions of degradation rate and net protein escape on reducing sugars were not significant ($P > .177$), quadratic ($P = .025$) and cubic ($P = .018$) relationships of ADIN to reducing sugars were significant (Figure 3A). Linear regressions of degradation rate on reducing sugars (Figure 3B) were significant ($P < .001$) with oven and steam heating of both unshredded and shredded alfalfa hays. Linear regressions of net protein escape on reducing sugars (Figure 3C) were significant with oven heating of both hays ($P < .001$) but not with steam heating of either hay ($P > .122$). These regressions indicate a direct but complex interaction of reducing sugars with protein and the effect of this interaction on resistance to ruminal degradation. The relationship of all three variables with reducing sugars was clear with oven-heated hay (open symbols; Figure 3 A, B, and C). However, the very rapid formation of ADIN, the rapid disappearance of reducing sugars with steam heating (Figure 3A), and our discounting of net protein escape for ADIN may explain the poorer corre-

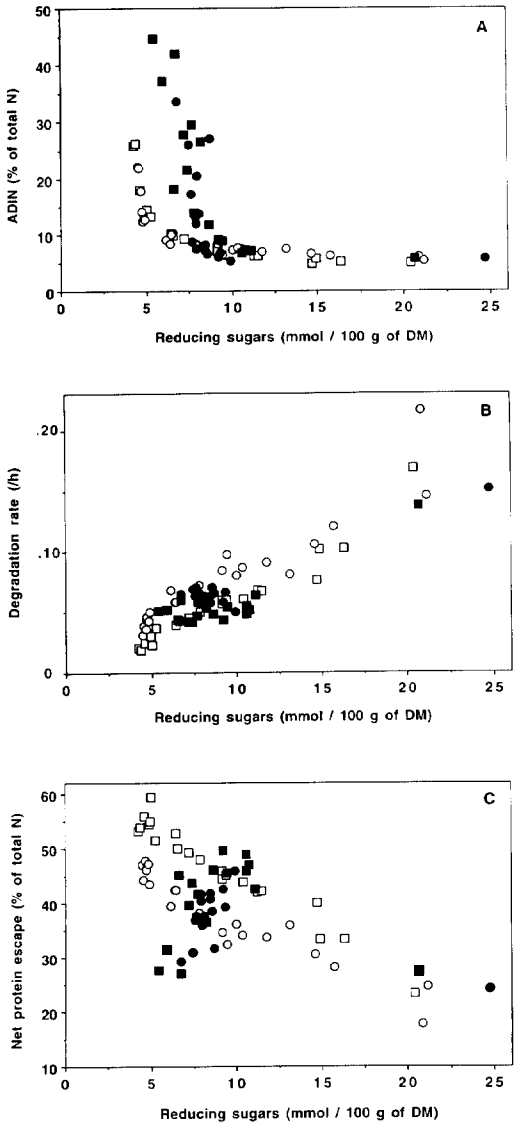

Figure 3. Relationships of A) ADIN, B) ruminal in vitro protein degradation rate, and C) net protein escape (total escape minus ADIN) to reducing sugar concentrations in oven-heated alfalfa hay that was either unshredded (○) or shredded (□) and steam-heated alfalfa hay that was either unshredded (●) or shredded (■).

lations for the steam-heated hays (closed symbols; Figure 3, A, B, and C).

The general doubling of ruminal protein protection with each 10°C increase in temperature reported by Faldet et al. (9) with heat treatment of whole soybeans also was observed with alfalfa hay. Degradation rates and net ruminal protein escapes for unshredded and shredded hays were essentially equal after 120 min at 140°C, 60 min at 150°C, and 30 min at 160°C of oven heating (Table 5). The pattern for degradation rate was not as clear with steam treatment, but net protein escapes were comparable for unshredded and shredded hays after 60 min at 100°C, 30 min at 110°C, and 15 min at 120°C of steam heating (Table 5).

Maximal net protein escapes were obtained with oven heating after 120 min (the longest heating time) at 130, 140, and 150°C and after 30 min at 160°C (Figure 1G). Although net protein escape was maximal with shredded hay after 60 min of steam heating at 100°C, maxima were at 15 to 30 min for all other treatments (Figure 2G). Optimal oven treatment was 120 min at 150°C (mean net ruminal protein escape for unshredded and shredded hay = 52.5%), but treatment for 60 min at 160°C was nearly equivalent. Optimal steam treatment by the same criterion was 30 min at 100°C (mean net protein escape = 45.9%), but nearly identical net protein escapes were obtained at 60 and 120 min at 100°C and after 15 min at 110 and 120°C. The broad optimum of 30 to 120 min at 100°C of steam heating suggests that it might be a robust practical treatment. Subsequently, we used low temperature steam heat to protect protein in alfalfa hay fed to lactating dairy cows (6).

Proportion of TN in ADIN was used in these studies as the criterion of overheating; higher temperatures and longer treatments substantially increased in ADIN (Figures 1C and 2C). Heat treatment of soybeans (9) gave rise to little ADIN until extensive heating was applied. However, Faldet et al. (9, 10) found that ADIN was a poor index of overheating because available lysine declined more rapidly than ADIN increased during heat treatment of soybeans. Available lysine decreased rapidly with time of autoclaving of cottonseed meal (7). Although not determined in our study, available lysine would be a valuable adjunct in further experiments on heat treatment of alfalfa hay.

TABLE 5. Mean composition of unshredded and shredded alfalfa hays that were oven treated at 140, 150 and 160°C for 120, 60, and 30 min, respectively, or steam heated at 100, 110, and 120°C for 60, 30, and 15 min, respectively.

Type of heat and forage treatment	Temperature	Time	NDF	Reducing sugars	ADIN	Degradation rate	Net escape
	(°C)	(min)	(% of DM)	(mmol/100 g of DM)	(% of TN)[1]	(/h)	(% of TN)
Oven							
Unshredded	140	120	52.2^{b}	6.5^{a}	9.9^{b}	.058^{a}	43.3^{b}
	150	60	54.8^{a}	4.9^{b}	12.7^{a}	.050^{a}	43.6^{b}
	160	30	53.5^{a}	4.8^{b}	12.3^{a}	.046^{a}	46.0^{b}
Shredded	140	120	49.6^{c}	6.5^{a}	10.2^{b}	.039^{b}	52.8^{a}
	150	60	53.5^{a}	5.2^{ab}	13.3^{a}	.037^{b}	51.4^{a}
	160	30	54.2^{a}	4.9^{b}	13.4^{a}	.032^{b}	54.4^{a}
Steam							
Unshredded	100	60	54.7^{ab}	8.6^{c}	7.1^{bc}	.063^{ab}	40.8^{b}
	110	30	54.3^{bc}	8.5^{c}	6.6^{c}	.070^{a}	38.6^{b}
	120	15	53.2^{bc}	8.4^{c}	8.2^{bc}	.057^{ab}	41.8^{b}
Shredded	100	60	52.6^{c}	10.6^{a}	6.8^{c}	.049^{b}	48.9^{a}
	110	30	55.9^{a}	9.4^{b}	8.8^{b}	.054^{b}	45.6^{a}
	120	15	55.6^{a}	8.6^{b}	11.8^{a}	.049^{b}	46.1^{a}

^{a,b,c,d}Means within columns for each type of heat treatment (oven or steam heating) with differing superscripts differ ($P < .05$). Probability of significant temperature-time effects was $P < .001$ for all traits.

[1]TN = Total N.

CONCLUSIONS

Oven heating or steam heating of alfalfa hay decreased protein degradation rate and increased net protein escape as measured using a ruminal in vitro system. However, heat treatment also increased fiber and especially ADIN. Increased protein protection with heat treatment may be explained by interaction of reducing sugars and protein. Optimal oven treatment, as indicated by maximal increase in net ruminal protein escape (total escape minus ADIN-bound CP), was 120 min at 150°C or 60 min at 160°C. Steam heating at 100°C for 30 to 120 min gave only slightly lower net ruminal protein escapes. Steam heating at temperatures greater than 110°C gave rise to rapid formation of very high percentages of ADIN. Low temperature steam heating may be a practical method to increase ruminal escape of protein in alfalfa hay.

ACKNOWLEDGMENTS

The authors gratefully thank D. B. Ricker, M. A. Elliott, and M. C. Becker for their excellent technical assistance and R. R. Smith and P. M. Crump for assisting with statistical analysis.

REFERENCES

1 Association of Official Analytical Chemists. 1980. Page 125 *in* Official Methods of Analysis. 13th ed. AOAC, Washington, DC.

2 Broderick, G. A. 1985. Alfalfa silage or hay versus corn silage as the sole forage for lactating dairy cows. J. Dairy Sci. 68:3262.

3 Broderick, G. A. 1987. Determination of protein degradation rates using a rumen in vitro system containing inhibitors of microbial nitrogen metabolism. Br. J. Nutr. 58:463.

4 Broderick, G. A., and W. M. Craig. 1980. Effect of heat treatment on ruminal degradation and escape, and intestinal digestibility of cottonseed meal protein. J. Nutr. 110:2381.

5 Broderick, G. A., D. B. Ricker, and L. S. Driver. 1990. Expeller soybean meal and corn by-products versus solvent soybean meal for lactating dairy cows fed alfalfa silage as sole forage. J. Dairy Sci. 73:453.

6 Broderick, G. A., J. H. Yang, and R. G. Koegel. 1993. Effect of steam heating alfalfa hay on utilization by lactating dairy cows. J. Dairy Sci. 76:165.

7 Craig, W. M., and G. A. Broderick. 1981. Effect of heat treatment on true digestibility in the rat, *in vitro* proteolysis and available lysine content of cottonseed meal protein. J. Anim. Sci. 52:292.

8 Faldet, M. A., and L. D. Satter. 1991. Feeding heat-treated full fat soybeans to cows in early lactation. J. Dairy Sci. 74:3047.

9 Faldet, M. A., L. D. Satter, and G. A. Broderick. 1992. Determining optimal treatment of soybeans by measuring available lysine chemically and biologically with rats to maximize protein utilization by ruminants. J. Nutr. 122:151.

10 Faldet, M. A., V. L. Voss, G. A. Broderick, and L. D. Satter. 1991. Chemical, in vitro, and in situ evaluation of heat-treated soybean proteins. J. Dairy Sci. 74: 2548.

11 Goering, H. K., and I. L. Lindahl. 1975. Growth and metabolism of sheep fed rations containing alfalfa hay or dehydrated alfalfa. J. Dairy Sci. 58:759.(Abstr.)

12 Goering, H. K., and P. J. Van Soest. 1970. Forage Fiber Analyses (Apparatus, Reagents, Procedures, and Some Applications). Agric. Handbook No. 379, ARS-USDA, Washington, DC.

13 Goering, H. K., and D. R. Waldo. 1978. The effects of dehydration on protein utilization in ruminants. Page 277 *in* Proc. 2nd Int. Green Crop Drying Congr. Br. Assoc. Green Crop Driers, Tunbridge Wells, Engl.

14 Hong, B. J., G. A. Broderick, R. G. Koegel, K. J. Shinners, and R. J. Straub. 1988. Effect of shredding alfalfa on cellulolytic activity, digestibility, rate of passage, and milk production. J. Dairy Sci. 71:1546.

15 Koegel, R. G., K. J. Shinners, F. J. Fronczak, and R. J. Straub. 1988. Prototype for production of fast-drying forage mats. Appl. Eng. Agric. 4:126.

16 National Research Council. 1989. Nutrient Requirements of Dairy Cattle. 6th rev. ed. Update. Natl. Acad. Press, Washington, DC.

17 Price, S. G., L. D. Satter, and N. A. Jorgensen. 1988. Dehydrated alfalfa in dairy cow diets. J. Dairy Sci. 71: 727.

18 Robertson, J. B., and P. J. Van Soest. 1981. Page 123 *in* The Analysis of Dietary Fiber in Foods. W.P.T. James, and O. Theander, ed. Marcel Dekker, New York, NY.

19 Roffler, R. E., and L. D. Satter. 1986. Evaluation of alfalfa preserved as high moisture silage, low moisture silage, or dehydrated pellets. J. Dairy Sci. 69 (Suppl. 1):142.(Abstr.)

20 SAS® User's Guide: Statistics, Version 5 Edition. 1985. SAS Inst., Inc., Cary, NC.

21 Sherrod, L. B., and A. D. Tillman. 1964. Further studies on the effects of different processing temperatures on the utilization of solvent-extracted cottonseed protein by sheep. J. Anim. Sci. 23:510.

22 Steel, R.G.D., and J. H. Torrie. 1980. Principles and Procedures of Statistics. McGraw-Hill, New York, NY.

23 Tagari, H., H. Ascarelli, and A. Bondi. 1962. The influence of heating on the nutritive value of soyabean meal for ruminants. Br. J. Nutr. 16:237.

24 Technicon Industrial Systems. 1973. Lactose in milk. Technicon Ind. Method No. 120-71A, Technicon Ind. Syst., Tarrytown, NY.

25 Voss, V. L., D. Stehr, L. D. Satter, and G. A. Broderick. 1988. Feeding lactating dairy cows proteins resistant to ruminal degradation. J. Dairy Sci. 71:2428.

26 Yu, Y. 1976. Relationship between measurements of heating and acid detergent insoluble nitrogen in heat-damaged fresh alfalfa, haylage and hay. J. Dairy Sci. 59:1845.
27 Yu, Y. 1977. Effect of heating of forages on quantitative changes of acid detergent insoluble nitrogen. J. Dairy Sci. 60:1813.
28 Yu, Y., and D. M. Veira. 1977. Effect of artificial heating of alfalfa haylage on chemical composition and sheep performance. J. Anim. Sci. 44:1112.